NMR Characterization of Dihydrosterculic Acid and Its Methyl Ester

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ABSTRACT: Cyclopropane FA occur in nature in the phospholipids of bacterial membranes, in oils containing cyclopropene FA, and in *Litchi sinensis* oil. Dihydrosterculic acid (2-octyl cyclopropaneoctanoic acid) and its methyl ester were selected for ^{1}H and ^{13}C NMR analysis as compounds representative of cyclopropane FA. The 500 MHz ^{1}H NMR spectra acquired with $CDCl_3$ as solvent show two individual peaks at –0.30 and 0.60 ppm for the methylene protons of the cyclopropane ring. Assignments were made with the aid of 2D correlations. In accordance with previous literature, the upfield signal is assigned to the *cis* proton and the downfield signal to the *trans* proton. This signal of the *trans* proton is resolved from the peak of the two methine protons of the cyclopropane ring, which is located at 0.68 ppm. The four protons attached to the two methylene carbons α to the cyclopropane ring also show a split signal. Two of these protons, one from each methylene moiety, display a distinct shift at 1.17 ppm, and the signal of the other two protons is observed at 1.40 ppm, within the broad methylene peak. The characteristic peaks in the ^{13}C spectra are also assigned.

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FA containing a cyclopropane structure (Fig. 1; shown as methyl esters), the most common of which contain 17 and 19 carbons, occur in seed oils such as Sterculiaceae, Malvalaceae, Bombaceae, and Tiliaceae that contain cyclopropene FA, and also frequently in bacterial cell membranes (1). Among these compounds are dihydrosterculic acid (x = 7, y =7 in Fig. 1; systematic name 2-octyl cyclopropaneoctanoic acid; sterculic acid is the corresponding cyclopropene FA), lactobacillic acid (x = 9, y = 5 in Fig. 1), and dihydromalvalic acid (x = 6, y = 7). Dihydrosterculic acid has also been reported in significant amounts (41%) in *Litchi sinensis* (lychee) seed oil (2), and its isolation and absolute configuration have been reported (3).

Numerous authors (3–12) have reported not only on the synthesis of cyclopropane FA but also analytical data for these compounds, with the signals of the methylene protons in the ^{1}H NMR spectra being assigned early, including distinguishing the *cis* and *trans* protons (4,5). Selected ^{1}H NMR data contained in the literature are compiled in Table 1. The ^{1}H NMR analytical data for the salient signals do not always agree, and in some cases assignments are incomplete. Although the ^{13}C NMR signals of cyclopropenoid FA have been assigned (13,14), those of cyclopropane FA were reported but no assignments made. Therefore, in light of the significant interest in long-chain compounds containing cyclopropane moieties, commercially available dihydrosterculic acid and its methyl ester were selected for NMR analysis as representatives of the class of cyclopropane FA. Full ^{1}H and ^{13}C NMR data are reported along with assignments of key peaks.

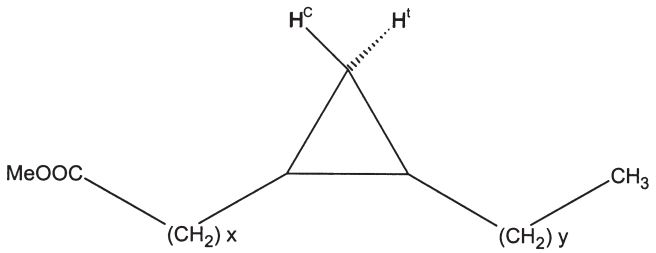

FIG 1. General structure of *cis*-cyclopropane FAME. The protons of the methylene unit in the cyclopropane are identified as *cis* and *trans* by the labels H^c and H^t, respectively.

EXPERIMENTAL PROCEDURES

Methyl dihydrosterculate and dihydrosterculic acid (synthetic material) were purchased from Matreya LLC (Pleasant Gap, PA). NMR spectra were acquired with $CDCl_3$ as solvent (approximately 40 mg sample dissolved in about 1 mL solvent) on a Bruker (Billerica, MA) Avance 500 spectrometer operating at 500 MHz (^{1}H) or 125 MHz (^{13}C). All chemical shifts are reported relative to the chloroform peak (7.29 ppm for ^{1}H NMR). Besides NMR, GC-MS analyses were carried out, using an Agilent Technologies (Wilmington, DE) 6890 GC with HP-5MS capillary column coupled to a 5973 mass selective detector, showing about 99% purity of the sample. The GC-MS results coincided well with the spectrum found by a library search (Wiley library) and literature data (3).

RESULTS AND DISCUSSION

Figure 2 depicts the ^{1}H NMR spectrum of methyl dihydrosterculate in the region of –0.5 to 2.5 ppm. The spectrum of the corresponding acid is virtually identical in this region. Expansions of the peaks at –0.30 and 0.60 ppm and the coupling constants for these signals are also shown in Figure 2.

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TABLE 1
Selected Literature Data on the ^{1}H NMR Spectra in the Range –0.5 to 2 ppm of FA or FAME Containing a Cyclopropane Ring

x; *y*; C1	Chemical Shifts[a]	Ref.
cis		
3; 17; $COOCH_3$	200 MHz; –0.38 (1H, m, *cis* H of CH_2 in cp ring); 0.54 (3H, m, *trans* H of CH_2, two C<u>H</u> in cp ring); 1.18 (32H, br, C<u>H</u>$_2$); 1.59–1.63 (4H, m, C<u>H</u>$_2$ of C4, C7), 1.67 (2H, m, C<u>H</u>$_2$ of C3)	7
4; 17; $COOCH_3$	200 MHz; –0.35 (1H, m, *cis* H of CH_2 in cp ring); 0.54 (3H, m, *trans* H of CH_2, two C<u>H</u> in cp ring); 1.24 (40H; (C<u>H</u>$_2$)$_{17}$$CH_3$, C<u>H</u>$_2$ of C4, C5); 1.61–1.67 (2H, m, C<u>H</u>$_2$ of C3)	7
4; 9; $COOCH_3$[b]	500 MHz; –0.32 (1H, H_a of CH_2, ddd, J = 5.2, 5.2, 4.6 Hz,); 0.55–0.60 (1H, m, H_b); 0.61–0.69 (2H, m, H of C6, C7); 1.08–1.49 (23H, m, positions 4–5 and 8–16, COO<u>H</u>); 1.64–1.71 (2H, m, 3 C<u>H</u>$_2$ of C3)	8[c]
7; 7; $COOCH_3$[d]	400 MHz; –0.33 (1H, m); 0.56 (1H, m); 0.64 (2H, m); 1.1–1.4 (24H, m); 1.62 (2H, m)	3
7; 7; COOH[e]	500 MHz; –0.36 (1H, q); 0.54 (1H, m); 0.63 (2H, br, s); 1.11 (2H, m); 1.30 (22H, m); 1.62 (2H, quint)	11
9; 5; COOH[f]	–0.28 (1H, q, CH, J = 3.7 Hz); 0.62 (3H, m, 3 × C<u>H</u>); 1.29–1.04 (22H, m, C_7H_{14}, C_4H_8); 1.61 (4H, m, 2 × CH_2)[g]	9
10; 2; $COOCH_3$	300 MHz; –0.34 (1H, m); 0.50–0.69 (3H, m); 1.25 (20H, br, s); 1.58 (2H, m)	10[c]
10; 2; COOH	400 MHz; –0.34 (1H, ddd, J_1 = 8.5 Hz, J_2 = 8.0 Hz, J_3 = 4.0 Hz); 0.54 (ddd, J_1 = 5.2 Hz, J_2 = 5.2 Hz, J_3 = 4.0 Hz); 0.64 (2H, m); 1.10–1.40 (20H, m); 1.62 (2H, quint, J_1 = 6.4 Hz)	12[c]
trans		
0; 14; $COOCH_3$	0.85 (5H; terminal CH_3; CH_2 in ring); 0.6 (methine at C2); 2.26 (methine at C3)	5
1; 13; $COOCH_3$	0.75 (C3 methine); 0.50 (C4 methine); 0.25 (CH_2 ring); 2.17 (2H at C2, dd)	5
2; 12–14; 0; $COOCH_3$	0.14–0.21 (CH_2 of ring); 0.34–0.40 (two methine protons)	5
7; 7; COOH	360 MHz; 0.11 (2H, t); 0.33 (2H, hept); 1.04–1.35 (24H, m); 1.61 (quint, 2H)	11
10; 2; $COOCH_3$	300 MHz; 0.35 (2H, m); 0.12 (2H, m); 1.24 (20H, br, s); 1.61 (2H, m)	10[c]
10; 2; COOH	400 MHz; 0.12 (2H, dd, J_1 = 6.5 Hz, J_2 = 6.5 Hz); 0.35 (dddd, J_1 = 17.2 Hz, J_2 = 8.9 Hz, J_3 = 6.3 Hz, J_4 = 4.5 Hz); 1.10–1.40 (20H, m); 1.60 (2H, quint, J_1 = 7.1 Hz)	12[c]
15, –[h]; $COOCH_3$	Five one-proton signals not assigned: 1.00, 0.90, 0.60, 0.40, –0.10	5

[a]All spectra were obtained using $CDCl_3$ as the solvent. Date for the terminal methyl group (around 0.88–0.90 ppm) of the FA chain is not included. br = broad, cp = cyclopropane, m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, hept = heptet.
[b]Absolute configuration given as 6*S*,7*R*.
[c]This reference gives similar data for other compounds not listed in this table but with the same absolute configuration.
[d]Methyl dihydrosterculate; absolute configuration given as 9*R*,10*S*.
[e]Dihydrosterculic acid.
[f]Lactobacillic acid; absolute configuration given as 11*S*,12*R*.
[g]Spectrometer type not given.
[h]Ring in terminal position, no terminal CH_3 group.

Table 2 lists the peaks in both the ^{1}H and ^{13}C NMR spectra as well as their assignments.

In previous literature, the ^{1}H NMR spectra of cyclopropane FA or FAME have been shown to exhibit several upfield signals assignable to the cyclopropane moiety (4,5). Corresponding data are compiled in Table 1. The data show that compounds with the cyclopropane ring in *cis* configuration show different shifts than those with *trans* configuration. An earlier study using molecular models and resulting interpretation showed that *trans* cyclopropane compounds do not show the upfield peak at about –0.3 to –0.35 ppm, because in this case the methylene protons in the cyclopropane ring would be equivalent (4). The same study (4) also details earlier disagreeing assignments of the protons in the cyclopropane moiety. The downfield peak at –0.3 to –0.35 ppm can be assigned to the *cis* proton of the methylene moiety in the cyclopropane ring. The majority of data for the *cis* compounds imply that a second peak at about 0.55–0.60 ppm exists, assignable to the *trans* proton of the cyclopropane methylene unit, which agrees with earlier literature (4,5). For the *trans* compounds, two data entries given in Table 1 agree on a peak at 0.11–0.12 ppm and a peak at 0.33–0.35 ppm, although the patterns differ.

However, some data show an overlap with a peak slightly downfield (0.60–0.70 ppm) caused by two protons. Also, the data do not give the same patterns of the various peaks. Furthermore, the methylene protons in the FA chain were usually reported to be contained in the usual broad methylene peak at 1.1–1.5 ppm, with the exception of a separate peak around 1.60–1.70 ppm caused by two protons (assigned to C3 in the chain, relative to the carboxyl moiety [7]). In one case (11), however, an unassigned separate multiplet at 1.11 ppm caused by two protons was reported.

To clarify this matter, in the present work, the ^{1}H (500 MHz) and ^{13}C spectra of dihydrosterculic acid and its methyl ester (Fig. 2) as well as 2D homo- and heteronuclear correlations were acquired with $CDCl_3$ as solvent. Peaks at –0.30 ppm, 0.60 ppm, and 0.68 ppm were observed, agreeing with some literature data (3,8,12), and correlated with ^{13}C NMR shifts at 10.93 for the methylene moiety of the cyclopropane ring as well as 15.75 and 15.78–15.79 for the methine car-

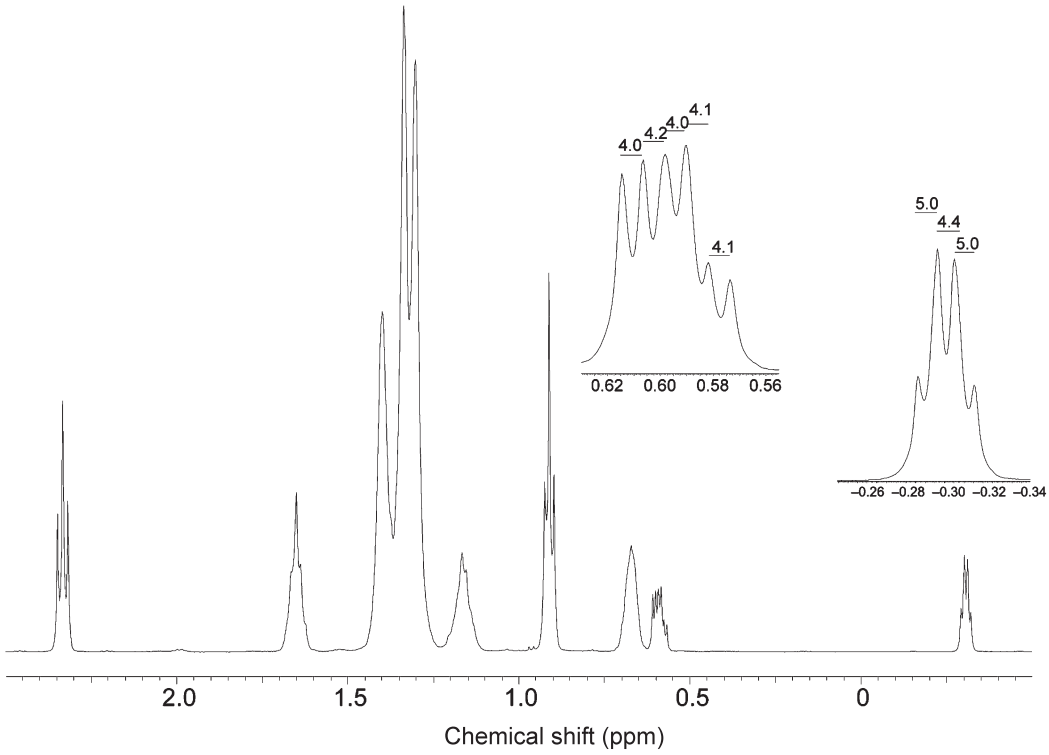

FIG 2. ^{1}H NMR spectrum of methyl dihydrosterculate (500 MHz, $CDCl_3$) in the region –0.5 to 2.5 ppm. The peaks at around 0.60 ppm and –0.30 ppm are expanded, and the coupling constants (Hz) are inscribed in these expansions.

bons. In other reports (3,7,11–12), the two peaks observed in the present work at 0.60 and 0.68 ppm apparently were not resolved. In the present work, 2D homonuclear correlation showed that the peaks at –0.30 and 0.60 ppm are assigned to the two C3 protons of the cyclopropane ring—*cis* and *trans*, respectively—and that the two methine protons resonate slightly downfield at 0.68 ppm.

The methylene peaks at 1.65 ppm and 1.17 ppm need to be investigated also. In agreement with most data in Table 1, the peak at 1.65 ppm is caused by only two protons. Two-dimensional homonuclear correlation shows clear correlation with the triplet caused by the protons attached to C2, indicating that this signal is assigned to the C3 methylene protons. On the other hand, the peak at 1.17 ppm, which was not resolved or listed separately in the previous literature, with one exception (11), correlates with the signal of the methine protons at

TABLE 2
^{1}H NMR and ^{13}C NMR Peaks with Key Assignments of Dihydrosterculic Acid ($C_{19}H_{36}O_2$) and Its Methyl Ester ($C_{20}H_{38}O_2$)

	^{13}C-NMR		
^{1}H-NMR[a]	Acid	Methyl ester	Assignment
–0.3 (1H, *cis*; q), 0.6 (1H, *trans*; ddd)	10.93	10.93	CH_2 of cyclopropane ring
0.92 (3H; t)	14.11	14.11	terminal CH_3
0.67 (2H; br)	15.75, 15.79	15.75, 15.78	CH carbons and protons (C9, C10)
1.25–1.45 (22H; br)[b]	22.71	22.70	CH_2 α to terminal CH_3 (-$\underline{CH_2}$-CH_3; ω-2)
1.65 (2H)	24.70	24.98	MeOOC-CH_2-$\underline{CH_2}$ or HOOC-CH_2-$\underline{CH_2}$
1.17 (2H; br), 1.40	28.68, 28.74	28.68, 28.73	CH_2 α to cyclopropane ring
1.25–1.45 (22H; br)[b]	29.09	29.18	CH_2 at C4 (MeOOC-CH_2-CH_2-$\underline{CH_2}$-)
	29.30, 29.37, 29.43	29.31, 29.37, 29.45	CH_2
	29.70	29.69	CH_2; 2 carbons
	30.13, 30.23	30.13, 30.22	CH_2
	31.94	31.94	-$\underline{CH_2}$-CH_2-CH_3 (ω-3)
2.33 (t, 2H)	34.06	34.13	HOOC-$\underline{CH_2}$- or MeOOC-$\underline{CH_2}$-
3.70 (s, 3H; ester only)	—	51.40	COO$\underline{CH_3}$
—		174.31	$\underline{C}$OOH or $\underline{C}$OOCH$_3$

[a]All spectra were obtained in $CDCl_3$ at 500 MHz (^{1}H NMR). For abbreviations see Table 1.
[b]This integration value contains two protons (resonating at 1.40 ppm) attached to the carbons α to the cyclopropane ring; see signal at 1.17 ppm. Also, the range 1.25–1.45 ppm appears twice to reflect the different signals in ^{13}C NMR correlating with this signal range.

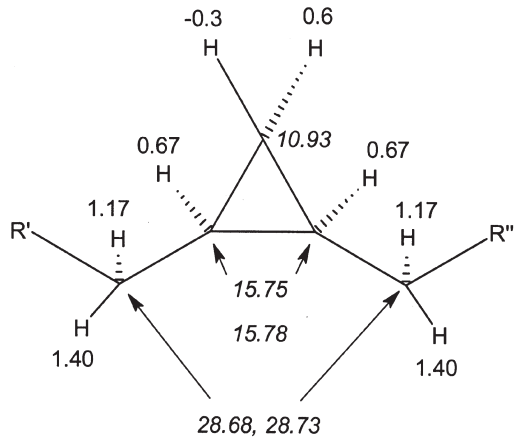

FIG 3. Assignments of chemical shifts (ppm; ^{13}C shifts are italicized) to the cyclopropane moiety in methyl dihydrosterculate. $R = -(CH_2)_6-COOMe$ and $R = -(CH_2)_6-CH_3$.

0.68 ppm and is therefore assigned to methylene protons · to the cyclopropane ring. However, the peak at 1.17 ppm is caused by two protons. In heteronuclear correlation, it correlates with ^{13}C NMR signals at 28.68 and 28.73 ppm. These two ^{13}C NMR peaks also correlate with the downfield region at 1.40 ppm, the broad methylene peak. The implication is that, likely due to shielding effects of the cyclopropane ring and similar to the differing shifts of the methylene protons in the cyclopropane ring, one proton each of the two methylene units · to the cyclopropane ring (at C8 and C11 of the FA chain) is responsible for the peak at 1.17 ppm. The downfield correlation is caused by the two other protons of these methylenes. A similar observation was made for FA with terminal cyclopentene moieties in which the signals of the methylene protons in the chain and the cyclopentene ring α to the "junction" carbon were split (15).

Table 2 contains not only information on the ^1H spectra but also on the ^{13}C spectra. The ^{13}C NMR signals not discussed above are assigned by general correlation with known data (14). The numerous methylene signals in the ^{13}C NMR spectra were not assigned to individual carbon atoms. Additionally, the assignments relating to the cyclopropane ring are depicted in Figure 3.

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